REVIEWS

CANCER ORIGINS

From melanocytes to melanomas

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Abstract | Melanomas on sun-exposed skin are heterogeneous tumours, which can be subtyped on the basis of their cumulative levels of exposure to ultraviolet (UV) radiation. A melanocytic neoplasm can also be staged by how far it has progressed, ranging from a benign neoplasm, such as a naevus, to a malignant neoplasm, such as a metastatic melanoma. Each subtype of melanoma can evolve through distinct evolutionary trajectories, passing through (or sometimes skipping over) various stages of transformation. This Review delineates several of the more common progression trajectories that occur in the patient setting and proposes models for tumour evolution that integrate genetic, histopathological, clinical and biological insights from the melanoma literature.

Solar elastosis
A degenerative change of the elastic fibres of the dermis induced by long-term exposure to UV radiation.

Precursor lesions
Melanocytic neoplasms that have an increased risk of progressing to melanoma.

Benign naevi
Circumscribed proliferations of melanocytes at the dermo-epidermal junction and/or in the dermis.

Dysplastic naevi
Clinically enlarged flat naevi, or naevi that show atypical cellular or architectural features microscopically.

Melanoma *in situ*
A proliferation of atypical melanocytes confined to the epithelial layer.

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Melanocytic neoplasms range from benign lesions, termed melanocytic naevi, to malignant ones, termed melanomas. All originate from melanocytes, which are neural crest-derived cells that, during development, colonize the skin, eye and, to a lesser degree, a broad range of other tissues throughout the body[1]. Melanocytes at these diverse sites can give rise to phenotypically diverse types of melanoma[2]. The most common types of melanoma in Caucasians are found on sun-exposed skin. These cutaneous melanomas can be broadly categorized by their origins from skin that is or is not chronically sun damaged (CSD and non-CSD melanomas, respectively; FIG. 1). CSD and non-CSD melanomas differ in their anatomical site of origin, degree of cumulative exposure to ultraviolet (UV) radiation, host age, mutation burden and types of oncogenic alteration[3–6]. CSD melanomas arise on skin that shows macroscopic and microscopic signs of long-term exposure to UV radiation, specifically marked solar elastosis. Thus CSD melanomas typically originate from the head, the neck and the dorsal surfaces of the distal extremities of older individuals (>55 years of age). They have a high mutation burden and are associated with neurofibromin 1 (*NF1*), *NRAS*, $BRAF^{nonV600E}$ or *KIT* mutations[2]. In contrast, non-CSD melanomas typically affect the more intermittently sun-exposed areas such as the trunk and proximal extremities of younger individuals (<55 years of age) that do not show marked solar elastosis. They are associated with a moderate mutation burden and a predominance of $BRAF^{V600E}$ mutations[2].

Primary melanomas are often found in association with different types of precursor lesions, ranging from benign naevi and dysplastic naevi to melanoma *in situ* (FIG. 2). Idealized progression models often imply a single path of evolution from naevus, to dysplastic naevus, to melanoma *in situ*, to invasive melanoma. However, the situation is more complex as there are multiple melanoma types, which can be linked to different precursor lesions (FIG. 3).

Fully evolved melanomas harbour multiple pathogenic mutations. The most recurrent somatic mutations in CSD and non-CSD melanomas affect genes in key signalling pathways that govern proliferation (*BRAF*, *NRAS* and *NF1*), growth and metabolism (*PTEN* and *KIT*), cell identity (AT-rich interaction domain 2 (*ARID2*)), resistance to apoptosis (*TP53*), cell cycle control (cyclin-dependent kinase inhibitor 2A (*CDKN2A*), which encodes $p16^{INK4A}$ and $p14^{ARF}$), and replicative lifespan (telomerase reverse transcriptase (*TERT*))[7–10]. The order in which these pathways become disrupted is incompletely understood and may be subject to variation. However, the presence of certain pathogenic mutations in precursor lesions and the association of these precursor lesions with specific types of melanoma provide important clues about the order in which mutations tend to accumulate (TABLE 1).

In this Review, we discuss the most common types of melanoma in Caucasians that originate from sun-exposed skin and propose evolutionary models of tumour progression by integrating genetic alterations at specific evolutionary stages with corresponding clinical and histopathological information. Thus, we delineate progression trajectories that capture the different scenarios of evolution found in patients.

Melanocytes

There are approximately 1,500 melanocytes per square millimetre of human epidermis, corresponding to nearly 3 billion cutaneous melanocytes in the skin of an average human[11]. Melanocytes represent a minority cell population within the basilar epidermis and divide infrequently — less than twice a year[12]. Their main function is to

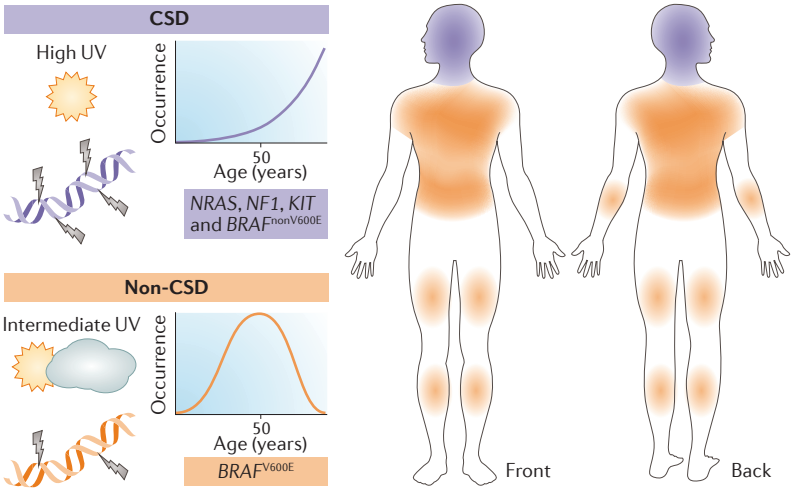

Figure 1 | **CSD and non-CSD melanomas are distinct subtypes of melanoma.** Melanomas on sun-exposed skin differ in clinical, histopathological and molecular aspects as a function of the degree of sun-induced damage of the skin on which they arise. Chronically sun-damaged (CSD) melanomas have higher mutation burdens and late age of onset, and occupy anatomical sites with the highest levels of sun exposure, such as the head and neck areas. Non-CSD melanomas present earlier in life, are often associated with naevi, have comparatively lower mutation burdens and occupy anatomical sites with intermediate levels of sun exposure. Each subtype of melanoma is characterized by distinct mutations, such as *BRAF*^{V600E} in non-CSD melanoma and *NRAS*, neurofibromin 1 (*NF1*), *KIT* and *BRAF*^{nonV600E} mutations in CSD melanoma.

provide melanin pigment to their neighbouring keratinocytes[13]. Their proliferation and pigment production is stimulated by UV radiation-induced DNA damage to keratinocytes, which subsequently secrete α-melanocyte stimulating hormone (αMSH) in a p53-dependent manner[14]. αMSH binds to the melanocortin 1 receptor (MC1R), which is expressed on melanocytes and induces synthesis of melanin, which melanocytes deliver to keratinocytes. Melanin is a complex macromolecule that scatters and absorbs UV radiation, and epidermal keratinocytes use it to protect their nucleus from UV radiation-induced DNA damage[15]. Epidermal melanocytes are thus part of a defence system against UV radiation-induced DNA damage.

Melanocytes are not confined to the epidermis. In the skin, they are also found in hair follicles, where they contribute pigment to the hair shaft[1]. Outside the skin, melanocytes are present in considerable numbers in the uveal tract of the eye and at lower densities in other tissues, such as the meninges and the anogenital tract; the melanocytic neoplasms that originate from these melanocytes are distinctly different in their clinical, histopathological and genetic make-up from cutaneous melanomas (see REF. 2 for a detailed review). For example, melanocytes in the anogenital and sinonasal mucosa give rise to melanomas that have a low mutation burden but highly rearranged genomes with numerous copy number changes, including multiple focused amplifications[3]. By contrast, in the eye, melanocytes within the choroid or iris give rise to uveal melanomas, which also have a low mutation burden and are characterized by frequent activating mutations in guanine nucleotide binding protein α q polypeptide (*GNAQ*) or *GNA11*, accompanied by inactivating mutations of the tumour suppressor gene BRCA1 associated protein 1 (*BAP1*) or mutations in splicing factor 3b subunit 1 (*SF3B1*)[16–18]. From these examples, among others, we speculate that these neoplasms arise from different cells of origin, which may be intrinsically different or may occupy different microenvironments, which changes their function and response to stimuli. We propose that these differences select for distinct somatic mutations that promote neoplastic growth. This is in part supported by experimental evidence. In a mouse model, expression of oncogenic mutant *Gnaq*^{Q209L} in melanocytes led to marked proliferation of melanocytes in the uveal tract and meninges, but had the opposite effect on epidermal melanocytes[19].

If distinct cells of origin are predisposed towards certain tumorigenic subtypes, it is likely that this cell-type variation also extends to the melanocytes in sun-exposed skin. The peculiar age distribution of neoplasms with *BRAF*^{V600E} mutations is striking (FIG. 1). Naevi with *BRAF*^{V600E} mutations arise in the first decades of life[20] and non-CSD melanomas (which commonly contain *BRAF*^{V600E} mutations) peak two to three decades later, dramatically dropping off in incidence after the sixth decade[21]. It has been proposed that cutaneous melanocytes of the trunk and proximal extremities may be especially susceptible to transformation following a *BRAF*^{V600E} mutation that occurs early in life, or that the susceptibility is confined to a specific subset of melanocytes, such as stem or progenitor cells, that may no longer exist or be accessible to mutagenesis later in life[2,22] (FIG. 3 step 1). The confinement to specific subsets of melanocytes may be linked to childhood and adolescence, when melanocyte density remains constant yet the body surface expands, implying that melanocytes must actively expand in number during this period. In conclusion, we encourage investigators to consider the possibility that melanocytes from different body sites or developmental stages have distinct vulnerabilities and/or predispositions to transformation. In particular, this possibility should be considered when working with primary melanocytes. For example, melanocytes derived from neonatal foreskin, a commonly used source, may not be able to fully recapitulate the biology of epidermal melanocytes from other sites.

Melanocytic naevus

Melanocytic naevi, termed naevi or naevus (singular) from here on, are benign proliferations of melanocytes, as they have a very low likelihood of progressing to melanoma. A broad range of different types of naevus have been described and linked to different types of melanoma[2], but we focus here on the most conventional type, the common acquired naevus or banal naevus. Caucasians on average have 25 such naevi on their skin that measure at least 2 mm in diameter[23,24]. Common naevi arise in the first two decades of life and have a tendency to regress again after the sixth decade[20]. Although individual naevi are unlikely to progress to melanoma, their high prevalence makes them contributors to a considerable portion of melanomas.

Basilar epidermis
The lower-most level of the epidermis.

Common acquired naevus
The most common form of benign naevus, which tends to arise on the skin during childhood or adolescence as a brown flat or raised mole.

Lentiginous growth pattern
The pattern of intra-epidermal growth of naevi and melanomas in which melanocytes are arranged mainly as individual units rather than as nests within the basilar epidermis.

Congenital growth pattern
The pattern of intra-dermal growth of naevi in which melanocytes are in the deeper dermis around appendages such as hair follicles, sweat glands and neurovascular bundles.

Several lines of evidence indicate that the common naevi that progress are associated with non-CSD melanomas (FIG. 3 step 2). Approximately 30% of non-CSD melanomas show areas representing a pre-existing common naevus[25], but some studies have found up to 90% of superficial spreading melanomas (a common histological presentation of non-CSD melanomas) to be associated with a naevus[26]. By contrast, associated precursor naevi are typically not found in CSD melanomas[26,27]. An evolutionary relationship between common naevi and non-CSD melanomas is also reflected in the high proportion of *BRAF*^{V600E} mutations found in both types of neoplasm[6,28], including melanomas specifically associated with an adjacent naevus component[29].

Histopathology. Acquired naevi can exhibit a lentiginous growth pattern or a congenital growth pattern. The latter category does not necessarily imply that the naevus was present at birth as in bona fide congenital naevi, but indicates that it has a similar growth pattern, in which melanocytes involve the deeper dermis, clustering around adnexal structures such as hair follicles. Lentiginous and congenital pattern naevi can be stratified as junctional, dermal or compound, depending on whether the constituent melanocytes are found at the dermo-epidermal junction, the dermis or both places, respectively. *BRAF*^{V600E} mutations seem to be present in most of these naevus types irrespective of their histological manifestations[28,30,31]; however, it will be important to more systematically characterize the genetics of these different subtypes of common naevus. If no genetic differences are found across the subtypes of common naevi, the different growth patterns may reflect differences in the type of melanocyte or the specific location (for example, the interfollicular epidermis or the superficial portion of the hair follicles) from which these melanocytes originated (FIG. 3 step 1).

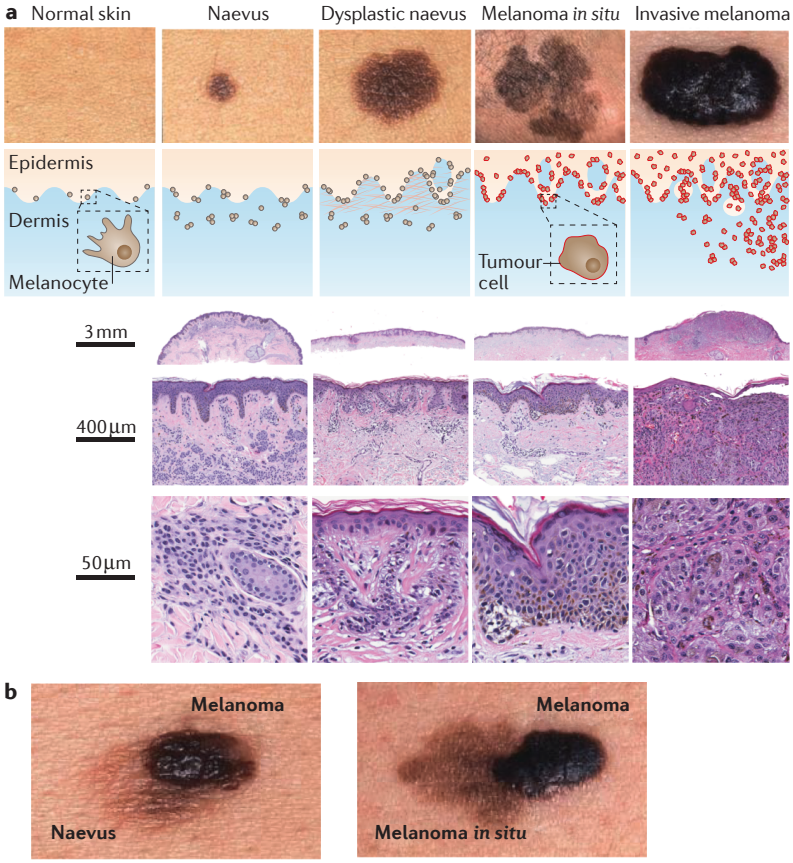

Figure 2 | **The morphological spectrum of melanocytic neoplasms. a** | Top row: clinical images showing a free-standing naevus, a dysplastic naevus, melanoma *in situ* and invasive melanoma. Second row: schematics illustrating the architectural features for each type of lesion as described in the main text. Rows 3–5: photomicrographs illustrating the representative histopathological features of each type of lesion as described in the main text. **b** | Clinical images showing combined neoplasms. As discussed in the main text, melanomas rarely pass through every histopathological stage.

Formation of naevi. Although previous work suggested that not all neoplastic melanocytes within naevi carried the *BRAF*^{V600E} mutation[32], recent studies using digital droplet PCR, mutation-specific immunohistochemistry or next-generation sequencing indicate that *BRAF*^{V600E} mutations are fully clonal, consistent with the notion that they are the initiating events[30,33]. Furthermore, ectopic expression of human *BRAF*^{V600E} in a zebrafish model is sufficient to form naevus-like proliferations[34]. These data suggest that *BRAF*^{V600E} mutations are sufficient for naevogenesis. In support of this notion, exome and targeted sequencing of genes frequently found in established melanomas have not revealed additional pathogenic mutations in common naevi, but have revealed that these lesions have comparatively low overall mutation burdens[30]. These observations strongly suggest that a single pathogenic mutation is sufficient to initiate the formation of a common naevus.

Naevus number and size are strongly influenced by an individual's constitutional genotype, as previous work comparing monozygotic and dizygotic twin pairs has shown[35], indicating that the frequency of initiating mutations, such as a *BRAF*^{V600E} mutation, and/or their penetrance is modulated by germline variation. The most significant naevus and melanoma susceptibility loci from population-based studies (reported in REF. 36) can be broadly divided into high-penetrance and low-penetrance alleles. High-penetrance alleles with low population frequencies are associated with more pronounced phenotypes such as larger naevi and markedly increased melanoma risk. These variants tend to affect genes residing in pathways that harbour frequent somatic mutations in sporadic melanomas (for example, the G1/S checkpoint or telomerase). Because they are associated with atypical naevi they will be discussed further below in the section on intermediate lesions. By contrast, low-penetrance alleles have markedly higher population frequencies and are associated with milder phenotypes such as lighter skin complexion, decreased tanning ability and a comparatively smaller melanoma risk. These alleles predominantly affect pigmentation genes, including *SLC45A2*, tyrosinase (*TYR*), *MC1R*, *OCA2* and agouti signalling protein (*ASIP*)[36]. Surprisingly, none of these variants has been individually linked to higher naevus count[36] even though skin complexion strongly associates with naevus count[37,38]. A factor partially explaining

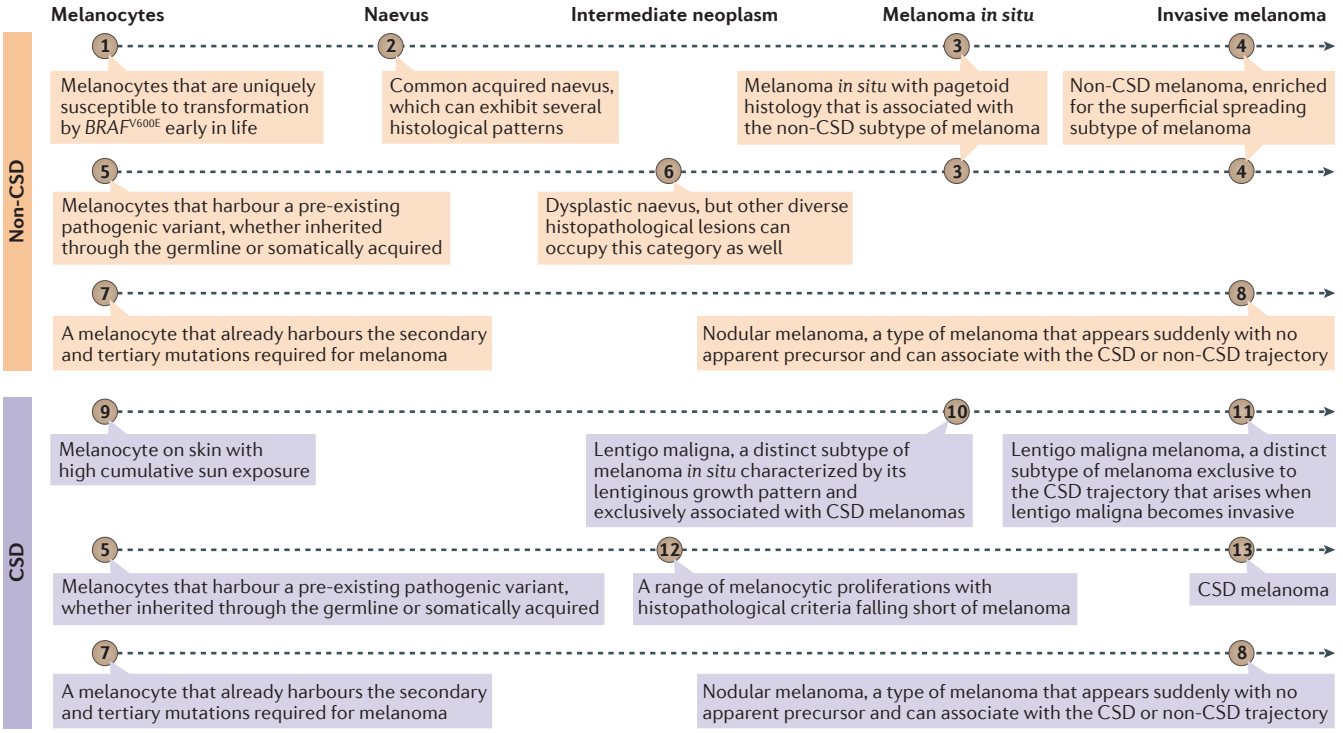

Figure 3 | **Common melanoma progression trajectories.** The relationship between clinically distinct melanomas, their precursors and their cells of origin as inferred from histopathological, clinical and genetic observations. Each entity (type of melanoma, precursor lesion or cell of origin) is described in detail in this Review article, where we refer to its depiction in the figure using the notation steps 1– 12, represented in the figure by circled numbers. CSD, chronically sun damaged.

this discrepancy may be that carriers of red hair alleles of *MC1R* typically have light complexion and freckles but have fewer visible naevi, despite an increased risk of developing melanomas on sun-exposed skin[39–41].

Germline variants in genes affecting complexion and the tanning response are likely to affect the mutation burden of cells on the surface of the skin in multiple ways and this may explain the mechanism by which certain variants promote carcinogenesis. Decreased pigmentation reduces UV shielding, contributing directly to mutagenesis. Variants of genes involved in pigmentation pathways can also affect the pheomelanin to eumelanin ratio[42]. Pheomelanin is a redder form of melanin that produces reactive oxygen species (ROS) upon exposure to UV radiation if not counterbalanced by sufficient amounts of the black eumelanin, and thus higher levels of pheomelanin can indirectly contribute to mutagenesis[43–45]. MC1R signalling not only induces eumelanin formation, but also promotes DNA repair and clearance of ROS, thus compounding the mutation-promoting effect of the loss-of-function variants of *MC1R* associated with light complexion and poor tanning ability[46].

Acquired naevi arise more commonly on sun-exposed anatomical sites and sun protection reduces their incidence, implicating UV radiation as a pathogenic factor in naevus formation[47–50] (FIG. 4). *BRAF*^{V600E} mutations generally result from a T→A transversion, which is not a common type of mutation induced by UV radiation-mediated DNA damage. However, the coexisting passenger mutations in naevi show clear signs of UV radiation-induced DNA damage, with a predominance of C→T transitions at dipyrimidine sites (which are susceptible to UV-induced damage)[30]. These observations implicate UV radiation as an initiating factor in naevus formation.

On the basis of the anatomical distribution of acquired naevi and a UV-associated mutation signature in most of their somatic mutations, we believe that UV radiation also contributes to the formation of *BRAF*^{V600E} mutations. The direct effects of UV radiation include the formation of cyclobutane pyrimidine dimers and [6–4] pyrimidine–pyrimidone adducts that manifest as CC→TT or (C/T)C→(C/T)T mutations[51], yet the *BRAF*^{V600E} hot-spot results from a T→A transversion. Such transversions are a minor byproduct of UV radiation and thus could still be a direct, albeit rare, consequence of exposure to UV radiation[52,53]. Error-prone DNA polymerases may also introduce these mutations after UV radiation exposure[52,53]. Alternatively, these mutations could result from indirect effects of UV radiation mediated by ROS. Although the exact mechanism needs to be resolved, the epidemiological and genomic evidence implies that UV radiation — directly or indirectly — contributes to the formation of *BRAF*^{V600E} mutations in cutaneous melanocytes.

Life of a naevus. After acquiring an initiating mutation, a melanocyte will undergo limited proliferation to form a naevus, before entering a state that has been described as "senescence-like"[54]. The strict definition

of cellular senescence invokes irreversible cell cycle arrest[55,56]. However, several observations indicate that at least some melanocytes within a naevus retain the ability to proliferate, arguing that they may not be strictly senescent. A small proportion of cells from explant cultures of naevi can proliferate for a short period of time[57,58]. Furthermore, a small proportion of naevus cells can be labelled with proliferation markers[59–61] and mitoses can occasionally be observed[62–64]. Naevus cells can also proliferate in response to certain stimuli, including UV radiation[65,66], incomplete removal[67], pregnancy[68] and immunosuppression[69]. Dermoscopic surveillance of naevi over time also indicates that naevi are morphologically changing rather than entirely stable[70]. Finally, phylogenetic data from sequencing studies reveal that some naevi evolve in multiple waves of expansion, indicating a more dynamic history of the melanocytes that form a naevus[30].

Clinically, most naevi do not change in size for many years. We hypothesize that the low levels of proliferation that occur within some naevi are counterbalanced by attritional factors that could be cell autonomous or non-cell autonomous. Apoptosis due to oncogenic stress has been shown to occur in melanocytic naevi[71], possibly counterbalancing low levels of proliferation. Also, several lines of evidence implicate the immune system as an attritional factor. Certain types of naevi are characterized by the presence of a chronic lymphocytic infiltrate and fibrotic changes reflecting chronic inflammation. This may be due to the fact that the naevus cells harbour oncogenic mutations known to induce immune-stimulatory and 'eat me' signals[72] that attract immune cells to eliminate them[73]. We speculate that in addition to these signals lymphocytes may also be attracted to the partially transformed melanocytes in naevi in response to the formation of neoepitopes from their somatic mutations. Further linking the immune system to regulation of naevus size and number, there is a germline variant in interferon regulatory factor 4 (*IRF4*) associated with higher naevus counts[36,74]. IRF4 is highly expressed in naevi and regulates inflammation in certain contexts, although the consequences of naevus-associated germline variants in *IRF4* are poorly understood. Also, there is a phenomenon known as eruptive naevi, in which numerous naevi suddenly increase in size or appear *de novo*, and this often occurs in patients who have become immunosuppressed owing to illness or therapy[75]. These eruptive naevi arise in anatomical sites that have previously been sun exposed and harbour oncogenic alterations similar to those of conventional naevi, indicating that the immunosuppression enables pre-existing melanocytes to increase in number[76]. Finally, common naevi tend to involute and then disappear after the age of 50[77], possibly indicating that the balance between slow proliferation and attrition tips in favour of naevus cell attrition.

Intermediate neoplasm (dysplastic naevus)

There has been a long-standing debate over the existence of a category of melanocytic neoplasm with a malignant potential that is intermediate between that of common naevi and that of unequivocal melanoma. This grey zone of neoplasms contains lesions with overlapping benign and malignant histopathological features (FIG. 3 steps 6 and 12), which prevent pathologists from issuing an unequivocal diagnosis and often results in considerable diagnostic variation between different observers. It also contains the contentious category of lesions known as dysplastic naevi[78,79], which will be at the centre of our subsequent discussion.

Table 1 | **Common mutations and their role during melanoma progression**

Pathway	Gene	Mutation	Subtype*	Progression phase‡	Role
MAPK	*BRAF*	V600E	Non-CSD	Naevi	Initiation
	BRAF	V600K, K601E and G469A, among other clustered nonV600E alterations	CSD	Intermediate and MIS lesions	Initiation
	NRAS	Q61R and Q61K, among other less common alterations affecting codon 61 or 12	CSD	Intermediate and MIS lesions	Initiation
	NF1	Disabling mutations occurring throughout the gene and deletions	CSD	MIS	Initiation
Telomerase	*TERT*	Promoter mutations affecting hg19 coordinates 1,295,228 or 1,295,250, among less common, nearby mutations	CSD and non-CSD	Intermediate and MIS lesions	Progression
RB	*CDKN2A*	Deletions and disabling mutations occurring throughout the coding region	CSD and non-CSD	Invasive melanoma	Progression
Chromatin remodelling	*ARID1A*, *ARID1B* and/or *ARID2*	Disabling mutations occurring throughout the protein	CSD and non-CSD	Invasive melanoma	Progression
PI3K	*PTEN*	Disabling mutations occurring throughout the protein and deletions	Non-CSD	Thicker invasive melanomas	Advanced progression
p53	*TP53*	Disabling mutations occurring throughout the protein	CSD	Thicker invasive melanomas	Advanced progression

ARID, AT-rich interaction domain; *CDKN2A*,cyclin-dependent kinase inhibitor 2A; CSD, chronically sun damaged; MIS, melanoma *in situ*; *NF1*, neurofibromin 1; *TERT*, telomerase reverse transcriptase. *Subtype refers to the melanoma subtype(s) predominantly associated with the mutation. ‡Progression phase refers to the earliest progression phase at which the mutation typically occurs.

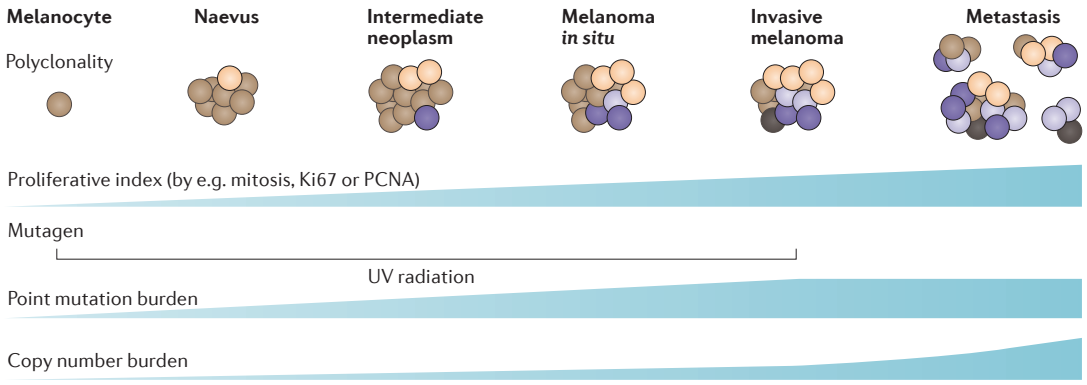

Figure 4 | **Biological characteristics of melanocytic neoplasms across the morphological spectrum.** Melanocytic neoplasms become more proliferative and polyclonal as they evolve. Most point mutations, which are caused by ultraviolet (UV) radiation, accumulate before the transition to invasive melanoma. Once melanomas become invasive, copy number alterations become more prevalent. PCNA, proliferating cell nuclear antigen.

Dysplastic naevus controversies. It is well established that certain melanoma kindreds have numerous enlarged naevi, a trait that cosegregates with their melanoma risk[80,81]. These naevi were claimed to have distinct clinical and histopathological features and have been termed dysplastic naevi. Similar lesions can also occur sporadically in individuals from families without a predisposition to melanoma[82]. Clinically, a dysplastic naevus is defined as a brownish patch of at least 5 mm in diameter, harbouring at least two of the following characteristics: variable pigmentation, asymmetry and/or irregular or indistinct borders[83].

Certain histopathological features have been associated with dysplastic naevi; however, these features correlate less well with the patient's melanoma risk and therefore remain a source of ongoing controversy[78]. The US National Institutes of Health (NIH) consensus conference has defined dysplastic naevi as lesions with architectural disorder, such as bridging of nests between rete ridges, subepidermal fibroplasia, dermal lymphocyte infiltration and lentiginous melanocytic hyperplasia with spindled or epithelioid melanocytes aggregating in nests of variable size[82]. Some classifications grade dysplastic naevi as mild, moderate or severe, assuming that the risk of progression to melanoma represents a spectrum that correlates with histopathological features[84]. However, interobserver agreement in diagnosing and grading dysplastic naevi is low[85–88]. Some clinicians treat incompletely excised lesions that are histopathologically diagnosed as dysplastic naevi as borderline malignancies with re-excision, whereas others consider them to be completely benign. As a consequence, dysplastic naevi are diagnosed and treated inconsistently[89].

The key question underlying the conundrum of dysplastic naevi is whether a biologically intermediate category between benign and malignant truly exists, or whether an intermediate category is merely a mirage, reflecting the limitations of histopathological assessment. Recent genetic analyses have shown that naevi unequivocally categorized as benign by multiple independent observers invariably had only a single driver mutation — $BRAF^{V600E}$ — whereas intermediate lesions, enriched for dysplastic naevi, had multiple driver mutations[30]. Driver mutations in intermediate lesions included mutations known to activate MAPK signalling and, additionally, *TERT* promoter mutations or hemizygous alterations of *CDKN2A*. Intermediate neoplasms also had overall mutation burdens higher than those of unequivocally benign lesions and lower than those of malignant lesions. These data lend strong genetic support for the existence of a biologically intermediate category, raising hope that the morphological criteria of these lesions can be refined in future studies.

Formation of dysplastic naevi. Clinical and histological evidence suggests that syndromic dysplastic naevi, that is, the multiple clinical dysplastic naevi associated with hereditary melanoma risk, arise *de novo* rather than from pre-existing common naevi. Syndromic dysplastic naevi tend to occur in families with a high penetrance of melanoma[78]. Examples of germline variants that confer a high penetrance of melanoma include *CDKN2A*, cyclin-dependent kinase 4 (*CDK4*), *TERT* and protection of telomeres 1 (*POT1*)[36,90–93], although the naevi associated with *TERT* and *POT1* variants have not been described. These same genes, or the pathways in which they operate, are frequently subject to somatic mutation in sporadic melanomas. The melanocytes of patients with such germline alterations thus have already inherited one of the multiple pathogenic variants found in melanomas (FIG. 3 step 5). Once these melanocytes acquire an initiating mutation that activates proliferation (presumably in the MAPK pathway), it may lead to a decreased ability to constrain proliferation, resulting in larger naevi. Thus, pathogenic alterations can pre-exist in a melanocyte without the formation of a discernible neoplasm and become revealed only in the context of an acquired mutation, on some occasions resulting in the evolution of the tumour skipping a histopathological stage.

In contrast to syndromic dysplastic naevi, dysplastic naevi can also occur sporadically, with an individual having only one, or a small number of dysplastic naevi. These also have an intermediate number of pathogenic alterations, but these mutations are probably all

Rete ridges
The 'pegs' of the epidermis that protrude into the underlying dermis.

Fibroplasia
A fibrotic change in the superficial dermis, which often has a lamellated appearance microscopically.

acquired somatically. This would align with the observation that these dysplastic naevi show a higher overall mutation burden than common naevi[30], as a higher mutation burden would be expected for multiple pathogenic mutations to arise. Interestingly, there seem to be additional differences in the genetic landscape of sporadic dysplastic naevi. Compared with the high prevalence of $BRAF^{V600E}$ mutations in common naevi, sporadic dysplastic naevi were found to be enriched for *NRAS* and $BRAF^{nonV600E}$ mutations[30] (FIG. 3 step 12). This further argues against the idea that dysplastic naevi arise from common naevi — as common naevi typically have $BRAF^{V600E}$ mutations — and suggests that some sporadic dysplastic naevi follow a separate evolutionary trajectory.

Life of an intermediate neoplasm. We hypothesize that the melanocytes in dysplastic naevi proliferate slowly, and that their proliferation is counterbalanced by attritional factors. Next-generation sequencing has revealed recurrent *TERT* promoter mutations in a significant proportion of dysplastic naevi[30]. This is an unexpected finding, as these lesions comprise only several hundred thousand cells. Assuming simple exponential growth, this would amount to fewer than 20 divisions of the founder cell, too low a number to allow for a substantial erosion of telomeres, the point at which TERT activation would be expected to become a selectable advantage. The divisional clock of these cells thus seems to have advanced much further than their cell number suggests; therefore, we hypothesize there must have been considerable cell attrition during their lives. In further support of this model, deep sequencing identified branching evolution of dysplastic naevi[30], which indicates that they arose through multiple waves of clonal expansion. One of the histopathological hallmarks of a dysplastic naevus is a chronic lymphocytic infiltrate in a fibrotic papillary dermis, possibly testifying to the chronic interaction between the neoplastic cells of the dysplastic naevus and the immune system. Dysplastic naevi can show occasional mitoses and some of their cells can be labelled with proliferation markers[64,94,95], thus suggesting a long-lasting balance between (slow) proliferation and attritional factors including the immune system. The involution of these naevi later in life could therefore be linked to attritional factors dominating and eliminating the lesion.

There is clear evidence that individuals with multiple dysplastic naevi are at increased risk of developing melanoma over their lifetime[83,96–98]. Nonetheless, the risk of progression of their individual dysplastic naevi seems to be very low[99]. Correlation of the genetic status with the histopathological and clinical characteristics of dysplastic naevi may help to refine the definition of the categories more accurately. Prospective and retrospective studies linking the genetic status of dysplastic naevi to outcome, such as eventual progression to melanoma, should also help to identify specific subsets of dysplastic naevi that may be closer to melanoma. In particular, it seems useful to distinguish between syndromic and sporadic dysplastic naevi.

Papillary dermis
The most superficial part of the dermis immediately beneath the epidermis. The papillae are dermal protrusions that reach towards the epidermis, between individual rete ridges.

Pagetoid growth
A growth pattern of melanoma in which enlarged melanocytes, singly or in nests, are scattered throughout all layers of the epidermis.

Nodule
A palpable protrusion of the skin.

Melanoma *in situ*

The term melanoma *in situ* refers to a proliferation of melanocytes with enlarged nuclei that grow in an irregular pattern entirely within the epidermis. This pattern is frequently encountered at the edges of invasive primary melanomas, but can also be seen in free-standing lesions without any invasive component. These latter lesions are staged as Tis, the earliest stage of melanoma put forth by the American Joint Committee on Cancer[100]. The survival rate is nearly 100% for completely resected melanoma *in situ*[101,102].

Histology and subtypes. Melanoma *in situ* can be a precursor lesion to both non-CSD and CSD invasive melanomas (FIG. 3). Two main patterns of melanoma *in situ* can be distinguished and they are associated with distinct evolutionary trajectories: those with a pagetoid growth pattern and those with a lentiginous growth pattern. The pagetoid growth pattern is associated with $BRAF^{V600E}$ mutations[4], thus placing these neoplasms on the trajectory of non-CSD or superficial spreading melanoma (FIG. 3 step 3). By contrast, the lentiginous growth pattern consists of melanocytes arranged as single units along the basilar epidermis. The lentiginous pattern is inversely correlated with $BRAF^{V600E}$ mutations, and therefore it is associated with the CSD trajectory (FIG. 3 step 10). Acral and mucosal melanomas also have a lentiginous form of melanoma *in situ*. The lentiginous form of melanoma *in situ* can extend over several square centimetres of skin over a period of several years before it becomes invasive and forms a nodule. This archetypical presentation of melanoma *in situ* on CSD skin is called lentigo maligna, and the invasive melanomas arising within them are termed lentigo maligna melanomas (FIG. 3 step 11). These lentiginous types of melanoma *in situ* predominantly affect older individuals.

***Precursors to melanoma* in situ.** Melanoma *in situ* on skin with chronic sun-induced damage (lentigo maligna) typically arises *de novo*, without any associated naevus that could have served as a precursor lesion[26]. The cell of origin of this melanoma *in situ* is not known (FIG. 3 step 9). The high mutation burden indicates that it is a superficially located melanocyte that could reside in the interfollicular epidermis. However, lentigo maligna frequently involves the hair follicles, making the follicular infundibulum or the bulge region of the hair follicle an alternative site for its cell of origin. Melanocytes in other adnexal structures such as the eccrine sweat glands have been shown to harbour the cell of origin of acral melanoma[103].

Melanomas arising in naevi, whether banal or dysplastic naevi, typically arise intra-epidermally, as melanoma *in situ* with pagetoid features[26] (FIG. 3 step 3). Mutation signatures implicate UV radiation-mediated mutagenesis of superficial melanocytes of the naevus as the predominant pathogenic mechanism that drives the progression of these naevi to melanoma, typically via melanoma *in situ* as an intermediate step[30].

Thin melanoma
Invasive melanomas can be subclassified by their thickness, measured as the distance in millimetres between the granular layer of the epithelium and its base. The term thin melanoma usually refers to stage T1 melanomas, which are less than 1 mm thick.

***Genetics of melanoma* in situ.** Deciphering the genetic composition of *in situ* melanomas has been technically challenging, because of their paucicellular nature. Sanger sequencing efforts to detect mutations in *BRAF*, *NRAS* and *TERT* from microdissected tissue have reported relatively uncommon mutations in melanoma *in situ*[29,104,105]. However, it is likely that the low sensitivity of Sanger sequencing underestimates the prevalence of mutations in melanoma *in situ* and thin melanoma. Efforts using assays with higher sensitivity have identified a significantly higher frequency of mutations affecting the MAPK signalling pathway, which occur mostly in *BRAF*, *NF1* and *NRAS*[30,106–108]. Melanoma *in situ* lesions also have a high frequency of *TERT* promoter mutations[30] and heterozygous *CDKN2A* alterations[30].

Formation and life of a melanoma in situ. *In situ* melanomas form from multiple pathogenic alterations accumulated over a considerable period of time, as reflected by their relatively late age of onset and slow development. This seems to be particularly notable for *NF1*-mutant neoplasms, which affect older patients[21,109]. Whereas most mutations affecting the MAPK pathway occur in a mutually exclusive pattern, *NF1* mutations commonly co-occur with other alterations in the MAPK pathway[110,111]. This indicates that loss of NF1 function is a weak activator of MAPK signalling, and thus these neoplasms must accumulate additional mutations over a considerable length of time to fully activate the pathway, explaining their particularly late age of onset.

Melanoma *in situ* lesions are more common on chronically sun-exposed anatomical sites and tend to have a high mutation burden with a strong mutation signature that is associated with UV radiation exposure. Also, many of the pathogenic mutations found in their driver genes, such as *TERT* promoter mutations and disabling mutations in tumour suppressors, exhibit signatures of UV radiation-induced DNA damage. Together, this implicates UV radiation as the main pathogenic factor in the development of melanoma *in situ* on sun-exposed skin (FIG. 4).

In situ melanomas can persist for many years before becoming invasive[112], indicating that invasive growth requires additional genetic alterations. We hypothesize that these additional genetic alterations may be needed to overcome dependencies from the microenvironment of the epidermis, which might explain why cells of melanoma *in situ* are difficult to culture and tend to require more growth factors than invasive melanoma cells[113–115]. However, escape from immune surveillance may also represent another requirement for transformation to invasive melanoma. It is notable that melanoma *in situ* lesions, despite their tendency not to form tumours for prolonged periods of time, are proliferative as assessed by increased Ki-67 labelling relative to common or dysplastic naevi[116]. This discrepancy, along with the finding of *TERT* promoter mutations in melanoma *in situ*, again indicates substantial turnover with proliferation partially offset by cell attrition, which might be eliminated by immune cells or cell-autonomous mechanisms.

Invasive melanoma

Once melanoma cells leave the epithelium of the epidermis and enter the subjacent mesenchymal tissue, such as the dermis or submucosa, the melanoma has become invasive. In contrast to many epithelial neoplasms, the ability to invade the dermis alone is not a malignant feature by itself, as most naevi are associated with melanocytes in the dermis. For melanomas, the risk of metastatic disease and death correlates with the depth of invasion[100]. Most invasive melanomas arise from a melanoma *in situ*, which, as discussed in the preceding section, can have different features, depending on the type of melanoma it is associated with. The notable exception is nodular melanoma (FIG. 3 step 8), which is defined by the absence of any discernable precursor lesion (discussed in more detail below).

Formation of invasive melanoma. Once a melanoma becomes invasive it 'inherits' the driver mutations activating the MAPK pathway, as well as *TERT* mutations that accumulated during earlier stages of progression. However, invasive melanomas display a high frequency of bi-allelic inactivation of *CDKN2A*, which is not seen in precursor lesions[30]. Immunohistochemistry (IHC) studies have also confirmed this observation, as negative reactivity for $p16^{INK4A}$, one of the two proteins encoded by the *CDKN2A* locus, correlates with invasive melanoma[117–119]. In animal models, $Nras^{Q61K}$ $Cdkn2a^{INK4A-/-}$ transgenic mice also develop invasive melanomas with short latency and high penetrance[120]. Together, these data imply that INK4A is a crucial barrier blocking the transition to invasive melanoma. It is possible, albeit uncommon, for invasive melanomas to retain $p16^{INK4A}$, suggesting that there are alternative routes to progression, most likely also overriding the G1/S checkpoint.

In addition to *CDKN2A* genetic alterations, mutations affecting members of the SWI/SNF chromatin-remodelling complex, particularly *ARID2* and *ARID1A*, emerge at the transition to invasive melanoma[30]. The SWI/SNF chromatin remodelling complex acts as a tumour suppressor in many cancers[121], including melanoma[7], although the underlying mechanisms remain unclear[122]. It has been proposed that SWI/SNF maintains genomic integrity[122,123], which is noteworthy, as the emergence of SWI/SNF mutations during progression of melanoma coincides with the appearance of widespread chromosomal aberrations[30] that are typically seen in fully evolved melanomas[3].

A subset of melanomas seemingly arise without any precursor lesion (FIG. 3 step 8). Although it is possible that these lesions have overwhelmed their precursors so that they are no longer detectable, some melanomas appear suddenly as nodular growths without discernible precursors. Previous studies have not identified significant differences in the spectrum of MAPK pathway activating mutations between these nodular melanomas and other melanoma subtypes[3]. Given the premise that formation of melanoma requires the occurrence of multiple independent mutations, nodular melanomas could arise either by mutations accumulating in rapid succession, or perhaps more likely, from a melanocyte (FIG. 3 step 7) that already harboured the secondary and even tertiary

genetic alterations, followed by the proliferation-initiating mutation, most likely one activating the MAPK pathway, as a later event[124].

The mutational processes that shape the genomic landscape of melanomas begin to shift once a melanoma becomes invasive (FIG. 4). There seems to be no further substantial increase in the burden of point mutations between the earlier, non-invasive portion of a given melanoma and its invasive portion[30]. This is consistent with UV radiation as the major factor in creating DNA sequence level alterations. Once cells grow deeper into the skin they evade the mutagenic effects of UV radiation. In contrast, the number of copy number alterations increases from melanoma *in situ* to invasive melanoma[125,126]. This indicates that other mutational processes are beginning to contribute to shaping the evolving melanoma genome.

Late-stage mutations in primary melanomas. *TP53* mutations are found in approximately 20% of metastatic melanomas[7,10]. Mutational studies of *TP53* in primary melanomas found lower mutation frequencies than reported for melanoma metastases[127–129], indicating that the mutations may arise later during the development of primary melanomas[130–133]. Similarly, *PTEN* mutations probably arise later in primary melanomas. *PTEN* mutations are more frequent in thicker primary melanomas and melanoma metastases[134,135]. An important caveat is that these genetic studies may have underreported the frequency of mutations in thinner primary melanomas because of stromal cell contamination. Nonetheless, for PTEN, IHC studies also found that protein loss correlates strongly with depth of invasion and risk of metastasis[136,137].

In summary, these data tentatively put bi-allelic loss of *CDKN2A* as an early event in invasive primary melanomas, whereas mutations of *TP53* and *PTEN* occur at later stages of progression, but further genetic analysis will be necessary to resolve the timing of *TP53* and *PTEN* mutations. In animal models, $BRAF^{V600E}$ cooperates with p53 loss of function to form melanomas in mouse[138] and fish[34] model systems and also cooperates with PTEN loss to form melanomas in mice[139]. The high penetrance and short latency in these model systems suggest that additional genetic alterations are not required for melanoma progression in these settings. By contrast, in human melanomas, inactivation of *CDKN2A* and activation of telomerase seem to be additional selected genetic alterations that occur before the alterations that affect *TP53* and *PTEN*.

Metastatic melanoma

Formation of melanoma metastases. Melanomas are termed metastatic once their cells have disseminated beyond the local site of the primary tumour and colonized other tissues. Similar to many solid tumours, melanoma metastases generally appear first in the lymph nodes of the draining area of the primary tumour, whereas distant metastases involving visceral sites tend to appear later. This presentation has led to the assumption that metastatic dissemination progresses serially from primary tumour to regional metastasis and finally to distant metastases. Pre-emptive removal of the regional lymph nodes is thus routinely performed with curative intent[140]. However, there are several observations suggesting that metastasis may arise in parallel rather than through a serial progression (FIG. 5). First, patients who undergo resection of their sentinel lymph node basin do not experience an extension of their life expectancy, contrary to what would be expected if the disease was regionally limited at some time during metastatic dissemination[141,142]. Second, circulating tumour cells are commonly found in patients who present with only regional metastasis, or even no metastases at all[143,144]. This indicates that regional nodes may not represent a boundary to dissemination, but that their involvement merely serves as a marker that metastatic dissemination has occurred, as has been shown for other cancers such as breast and endometrial carcinoma[145–148]. Finally, phylogenetic analyses of matched primary and metastatic lesions found patterns of parallel dissemination in most of the patients studied[149]. This study also revealed that melanoma metastases can be founded by multiple cells, either simultaneously or — perhaps more likely — by reseeding over a period of time. We speculate that the latter scenario would provide an explanation for the apparent head start of regional metastases. Because of their direct connection to the primary tumour through lymphatic channels, departing cells would have a significantly increased probability of landing at the same location, thereby increasing the number of founding cells compared with distant sites where repeated seeding is less likely.

There is evidence that even cells from melanoma precursors, such as those found in melanocytic naevi, are already capable of dissemination and limited colonization (FIG. 5). Small deposits of morphologically bland melanocytes are routinely found in lymph nodes removed from patients without any history of melanoma[150,151]; these have been termed 'nodal naevi'. Nodal naevi are especially common in the sentinel lymph node of patients with melanoma that has originated from naevi[152], presumably because naevus cells recurrently disseminated before the primary tumour had formed. As well as these histological observations, genetic studies have identified $BRAF^{V600E}$ mutations in nodal naevi[153], but not other genetic alterations found in melanomas[154], supporting the notion that these nodal naevi truly arise from melanocytic naevi in the skin. The phenomenon of a benign tumour giving rise to a metastatic deposit has been termed benign metastasis, and it is not restricted to melanocytic neoplasia — other examples include benign uterine leiomyomas[155], pleomorphic adenomas[156], fibrous histiocytomas[157], adenomyoepithelioma[158] and meningioma[159]. On the basis of these findings we hypothesize that metastatic dissemination is an inherent capability even of benign melanocytic neoplasms and is not an acquired feature of progression; however, bona fide melanoma metastases grow significantly larger and thus have an enhanced proliferative capacity and/or ability to colonize tissues or evade the immune system.

Approximately 4% of all melanomas arise as apparent metastases without a detectable primary tumour, and these are known as melanomas of unknown primary (MUP).

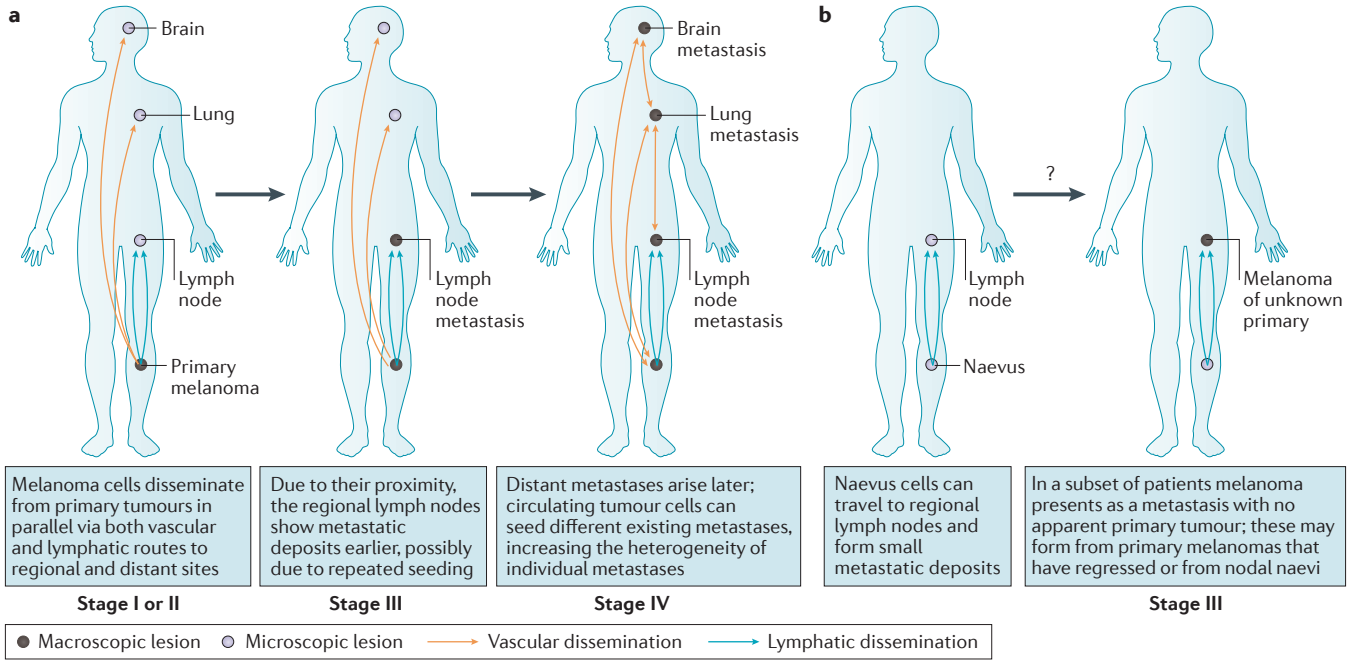

Figure 5 | **Models of melanoma metastasis. a** | Primary melanoma cells disseminate in parallel to local and distant sites throughout the body. Metastases are first evident in local lymph nodes, owing to their direct connection to the primary melanoma by lymphatic vessels, which could allow for repeated seeding of lymph nodes, accelerating the growth of nodal metastasis. Circulating tumour cells can seed different existing metastases and the primary tumour. **b** | A small proportion of metastases are found without an apparent primary tumour. The evolutionary history of these melanomas of unknown primary remains poorly understood, but we speculate that some may represent primary melanomas that arose from cells that disseminated from benign precursors such as naevi and acquired their secondary and tertiary oncogenic alterations after colonizing other sites.

Two-thirds of MUPs arise within lymph nodes and the rest arise at visceral sites[160]. MUPs have a high mutation burden with UV radiation-induced mutations, similar to melanomas with an identifiable cutaneous primary, indicating that they originated from sun-exposed skin[161]. Some may originate from primary melanomas that underwent spontaneous regression or were excised and misdiagnosed. However, we speculate that a subset originate from melanocytes that metastasized from naevi (FIG. 5). In this model, these melanomas would have acquired the additional mutations required for full transformation at the metastatic site, and thus they may be more akin to primary melanomas than to melanoma metastases. In line with this scenario is the clinical observation that MUPs tend to have a significantly better prognosis than bona fide metastatic melanomas with similar sites of involvement[160]. This model needs to be tested further; however, the cumulative evidence suggests that metastatic dissemination in melanocytic neoplasia can occur early, even before a melanoma has formed, but that formation of clinically detected metastases is dependent on additional genetic alterations (FIG. 5).

It is unresolved whether certain driver mutations promote departure from the primary melanoma and/or colonization of a new site during metastatic dissemination. Analysis of cohorts of matched primaries and metastases by exome sequencing has not identified recurrent driver mutations that are restricted to metastases[149,162–165]. However, anecdotal genetic evidence implicates activation of WNT signalling through activating mutations of β-catenin as a candidate, as different activating mutations of β-catenin were identified in separate metastases in a patient that could be traced back to subpopulations in the primary tumour[149]. Activation of WNT signalling also promotes metastasis in a mouse model of melanoma[166]. Although there may be instances in which pathogenic mutations promote metastatic dissemination, a conclusive pattern of recurrent alterations associated with metastatic progression has yet to emerge.

Life of a melanoma metastasis. Melanoma metastases tend to have the highest proliferative index of all stages of melanoma[167]. The genetic heterogeneity of an individual melanoma metastasis is expected to be lower than that of a primary melanoma because its population diversity is reduced during clonal expansion of a small number of founding cells. Nevertheless, subclones are detectable in metastases[149]. Mutations that confer resistance to targeted therapies, such as those directed against BRAF-V600E mutant proteins, can also lead to simultaneous emergence of resistant clones at many separate body sites[168]. As this type of relapse can arise within only a few months after an initial therapeutic response, it is likely that the cells carrying the resistance mutations pre-existed at multiple sites as minority subclone populations. Together, these observations support

a model in which metastases have the ability to reseed each other (FIG. 5). This model is further supported in a mouse model of melanoma[169] and in phylogenetic studies of human prostate cancers[170]. The clonal architecture of metastases thus does not necessarily have to reflect branching evolution within the metastasis itself, but can also reflect seeding from tumour deposits elsewhere in the body. Sites that have already been settled by other tumour cells may offer a more hospitable environment than uninvolved tissues. Reseeding adds considerable complexity to the diversity of metastatic clones and how resistance to therapy can evolve.

Conclusions

There are distinct subtypes of melanoma that differ in their cell of origin, anatomical site, role of UV radiation as a pathogenic factor, pattern of somatic mutations and type of precursor lesion. The two most common types of melanoma in Caucasians are CSD melanoma and non-CSD melanoma. The most common initiating mutation in non-CSD melanomas is *BRAF*^{V600E}. In the absence of other driver mutations, the *BRAF*^{V600E} mutation results in a limited expansion of melanocytes to form a common naevus. These naevi are stable lesions that are probably composed of a mixture of permanently arrested (senescent) cells and slowly proliferating cells that are offset by immune cell-mediated attrition. Rarely, these banal naevi progress to melanoma through acquisition of secondary and tertiary mutations such as *TERT* promoter mutations and bi-allelic loss of *CDKN2A*. By contrast, CSD melanomas have a different set of driver mutations, such as *NRAS*, *NF1* or *BRAF*^{nonV600E} mutations. They do not ordinarily originate from common naevi, but instead from melanoma *in situ* lesions or intermediate lesions.

Activation of the MAPK pathway is probably required to form a discernible melanocytic neoplasm. Constitutive activation of this pathway alone will only partially transform melanocytes, as additional alterations are necessary for full transformation. Telomerase activation by mutations in the *TERT* promoter region is a common and early event in the evolution of primary melanomas. Additional alterations, most prominently affecting the G1/S checkpoint, are required for melanoma formation. Mutations that promote proliferation ordinarily occur the earliest during the evolution of melanoma; however, inherited or somatically acquired alterations that disable later barriers to transformation, such as alterations that affect *TERT* or *CDKN2A*, can pre-exist in melanocytes, and in this scenario may enable tumour evolution to skip to a more advanced histopathological stage once a proliferation-inducing mutation occurs. There is emerging evidence that an intermediate stage of melanocytic neoplasia exists, in which tumour cells harbour more than one driver mutation but fewer than are required for full transformation. UV radiation is the predominant mutagen acting during all stages of melanoma evolution until the transition to invasive melanoma, when other mutagenic mechanisms, such as chromosomal instability, predominate. As UV radiation can no longer act as a mutagen on invasive cells, there may be positive selection for genetic alterations that increase chromosomal instability as a substitute mutator mechanism.

Our improving insight into the genetic evolution of melanomas from their cells of origin through different types of precursor lesion offers opportunities for improved diagnosis, earlier recognition of lesions at increased risk of progression and selective intervention at an earlier stage. The gold standard by which to assess the malignant potential of melanocytic neoplasms is histopathology, which has demonstrable limitations in accurately separating lesions with different risk potential[171]. Biomarkers that define individual progression steps are emerging and are expected to have an increasing role in assisting diagnostic classification. The crucial role of UV radiation in advancing the main types of melanocytic neoplasia in Caucasians from one evolutionary stage to the next has become unequivocally clear, providing an opportunity to further improve and refine public health campaigns for sun avoidance. Similar studies are needed to understand the evolution and mutator mechanisms in melanoma subtypes that are not related to UV radiation, such as acral and mucosal melanoma, which affect all world populations at comparable incidence.

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Competing interests statement
The authors declare no competing interests.

FURTHER INFORMATION
The Cancer Genome Atlas: http://cancergenome.nih.gov
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